 DOI 10.1002/ejlt.200800252 Eur. J. Lipid Sci. Technol. 2008, *110,* 1190–1194

Meeting Report

Highlights of the International Symposium on Plant Lipids, 18th ISPL 2008, Bordeaux July 20–25th, 2008

Plant lipids are of considerable interest in economic sectors such as agriculture, food, health and the cosmetics industry. In addition, the sector of renewable energies is showing increasing interest in the field of plant lipids.

The lipids of plants constitute the majority of the reserves of seeds from field crops like: soya, rape, sunflower, palm tree, and olive tree. Annual world production attains 120 million tonnes and represents a sales turnover of €8 billion. The oils extracted from these seeds have different properties according to their composition in lipids and fatty acids. Their uses will be varied according to their properties. They are destined for both food and non-food uses. The improvement of their properties and their diversity are the object of research undertaken by companies of international size covering a broad sector from the improvement of seeds, the production of diesters lubricants to the beauty care sector.

Concerning the food uses of vegetable oils, two aspects are important: on the one hand, productivity improvements to face the increasing demands of a world population in continuous growth and the needs of emerging countries and, on the other hand, the improvement of food value in order to decrease the cardiovascular and obesity problems encountered in western countries. To reach the first of these objectives, it is necessary to improve the output by agronomic or biotechnological methods. The second objective requires the modification of the properties of vegetable oils or the anticipated production of new oleaginous plants.

The presence of particular fatty acids like omega-3 and omega-6 and the presence of vegetable sterols in the oils of plants are the basis for many functional foods, as these lipids have shown to be beneficial in preventing cardiovascular diseases and decreasing the incorporation of cholesterol in the membranes of arteries.

Lipids are also highly valued by the cosmetics industry for their free anti-radicals properties (carotenes, flavanoides), and vegetable oils are used ever more extensively as the basis of solar creams and lotions. In the same way, the vegetable ceramides and inositols are the active molecules of new antiwrinkle creams. The field of application of vegetable lipids in the beauty care industry is only at its beginnings and its development is primarily limited by the current state of knowledge.

Our society is highly dependent on raw fossil materials whose reserves are not inexhaustible and whose prices are continually rising. To mitigate the impact of this situation, it has become a priority to find new sources of alternative and renewable energy. Oils from oleaginous field crops like sunflower and rape possess properties close to those of fossil oils, and it is therefore reasonably probable that alternative raw materials may be produced from vegetable oils. Diester is the first example, and the production of other materials like biolubricants, solvents, and inks is completely attainable.

Vegetable oils, and more generally the lipids of the plants, are of undeniable economic and social importance. New industrial investments linked in particular to the improvement of our society's health and to alternative energy solutions imply the prior development of knowledge of the metabolism of plant lipids. One of the decisive factors for this development is the diffusion and confrontation of ideas between the various research actors, and the series of International Symposia on Plant Lipids, ISPL. The 18th ISPL was held from 20 to 25 July 2008 at "La Cité Mondiale" in Bordeaux, France.

The ISPL 2008 is the continuation of a series of meetings which gathers the community of researchers specialised in plant lipids. The first symposium was organised in 1974 in Norwich, Great Britain, by T. Galliard over two and half days, and gathered 90 participants including 25 internationally renowned scientists.

The meeting gathered 270 researchers from 33 countries. Sixty nine oral communications and 136 posters have been presented during the 12 sessions of the symposium which have covered all the different aspects of the Plant Lipids field. Two researchers have been distinguished during this week. Professor S. Stymne (Université de Alnarp, Sweden) has received the T. Galliard medal for his work concerning the study on the lipid biosynthesis on oilseeds and for the manipulation of fatty acid composition by biotechnology. The first "P.K. Stumpf award" has been created to reward a promising young researcher; this award has been attributed to S. Baud (Institute J.P. Bourgin, Versailles, France) for his work concerning the lipid storage during the seed development.

Prof S. Stymne presenting the T. Galliard lecture.

S. Baud receiving the P. K. Stumpf award from J. Harwood.

As in previous ISPL symposia a lot of important data has been reported during the 2008 meeting in Bordeaux, a selection of the results presented during the different sessions is reported below.

Session 1: Fatty acids

The details of "unusual", *e.g.* hydroxyl or conjugated fatty acid formation remain elusive because these enzymes await structural characterization. However, soluble plant acyl-ACP desaturases have been studied in far greater detail but typically only catalyze desaturation reactions. By using a mutant of the castor acy-ACP desaturase that converts stearoyl-ACP to allylic alcohol (*E*)-10-18:1-9-OH *via* (*Z*)-9-18:1 intermediate, J. Shankilin (Brookhaven National Laboratory, New York, USA) has contributed to detailed mechanistic insights into factors that govern the highly-selective production of unusual fatty acids.

Another main result in this session has been reported by Bach *et al.* who has characterized the 3-hydroxyacyl-CoA dehydratase which was the last constituent unidentified of the acyl-CoA elongase which is responsible for the synthesis of very long chain fatty acids (VLCFA). They showed that Arabidopsis *PASTICCINO2* (*PAS2*) was able to restore the yeast 3-hydroxyacyl-CoA dehydratase *Phs1* cytokinesis defects and long chain base (LCB) accumulation; and that the *pas2-1* mutant was characterized by a general reduction of VLCFA pools in seed storage triacylglycerols (TGA), cuticular waxes and complex sphingolipids and by the accumulation of 3-hydroxy-acyl-CoA intermediates.

Session 2: Glycerolipids

Galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are predominant membrane constituents of chloroplasts and other plastids, and regarded to have important function in plastids. It has been previously shown that galactolipids are indispensable for proper chloroplast biogenesis, photosynthesis and moreover, embryogenesis in Arabidopsis. Using a knockout of *MGD1 (mgd1-2)* which causes significant decrease in both MGDG and DGDG, Ohta *et al.* found that the mutant showed a little developed thylakoid membrane and an activation of photosynthesis-related gene expression even under Pi-starved condition, although it has no photosynthetic activity. The result suggests strict requirement of MGDG particularly in photosynthesis.

Session 3: Sphingolipids, sterols, isoprenoids

Sphingolipids in plants as in yeast and animals are essential for important cellular functions such as endocytosis, protein transport, apoptosis and stress responses. They are characterized by a large degree of structural diversity and 200 different species exist including glucosylceramides (GlcCer) and glycosylinositolphosphoceramides (GIPC), the two major sphingolipid classes of plants. Cahoon *et al.* have undertaken studies to determine the functional significance of the C-4 hydroxyl group and the Δ8 double bond of LCB in Arabidopsis sphingolipids. By generation of mutants devoid of the C-4 hydroxy group, they have determined that this structural modification plays a central role in growth and in mediating sphingolipid metabolism. These mutants are severely dwarfed and do not progress from vegetative to reproductive growth. In addition, the total content of sphingolipids in these plants is increased by 2.5-fold relative to wild-type controls. This increase is due largely to the accumulation of sphingolipid species with C_{16} fatty acids, rather than the more typical very long-chain fatty acids. By contrast, mutants devoid of Δ8 unsaturation of LCB do not display reduced growth, suggesting that this structural modification is not critical for Arabidopsis when plants are maintained under non-stress conditions.

Session 4: Surface lipids – Cutin, waxes and suberin

The outer epidermal cell walls of plant shoots are covered by a cuticle, a thin hydrophobic structure that protects plants against desiccation, UV-light, pathogens and insects. The cuticle is composed of a cutin polyester matrix embedded in and covered with waxes. Even though major pathways for cuticular wax biosynthesis have been proposed, our knowledge of the enzymes involved in the biosynthesis pathway and the regulation of the cuticle synthesis is incomplete.

Kunst *et al.* have identified a cytochrome P450 gene named *MAH1*, and a bifunctional WS/DGAT gene designated *WSD1*. MAH1 and WSD1 were characterized by heterologous expression in yeast and *E. coli*, and by ectopic overexpression in Arabidopsis leaves. MAH1 is a mid-chain alkane hydroxylase and WSD a wax synthase. *CER1* gene which encodes a putative aldehyde decarbonylase involved in the wax alkane production.

Joubes *et al.* have reported data concerning the regulation of wax synthesis. They showed that the expression of *CER1* (encoding for a putative aldehydide decarbonylase) and the alkane formation are regulated in an ABA-dependent manner suggesting an important contribution of the cuticular waxes in the adaptive response of the plant in a situation of water deficit.

Important progress has also been reported concerning the cutin biosynthesis. Li *et al.* have studied double knockouts *gpat4/gpat8*, which are affected in glycerol-3-phosphate acyltransferases (GPAT), were strongly reduced in some cutin monomers and showed a steep increase in cuticle permeability. Overexpression of any of these acyltransferases in Arabidopsis increased the content of specific C_{16} and/or C_{18} cutin monomers, up to 80% in leaves and stems. These results identify the first acyltransferases involved in cutin synthesis.

Session 5: Seed oils and bioengineering of metabolic pathways

In recent years there has been tremendous interest in improving not only the total seed oil content of domesticated oilseed crops, but also in the knowledge-based engineering of new metabolic pathways into these crops for production of high-value specialty fatty acids.

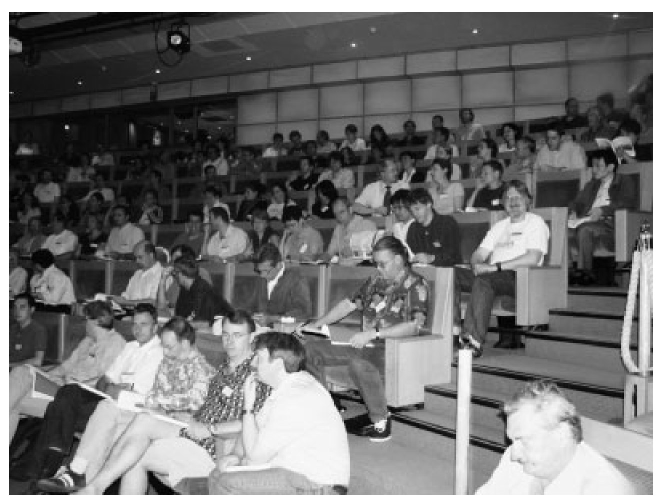
Several talks in this session highlighted recent advances in each of these areas. J. Dyer focused on the role of acyltransferases in the enrichment of unusual, industrially important fatty acids in seed oils, using the tung tree (*Vernicia fordii*) as a model system which accumulates oil contains high amounts of eleostearic acid. J. Dyer reported also the biochemical characterization of two diacylglycerol acyltransferases (DGAT1 and DGAT2) and indicated that DGAT2 played a more direct role in the channeling of eleostearic acid into tung oil. Moreover, the DGAT1 and DGAT2 proteins were located in distinct and separate regions of the endoplasmic reticulum (ER) suggesting that oil production in plant cells, and perhaps fatty acid channeling in particular, may take place in specific subdomains of ER. The importance of the DGAT2 enzyme family in the channeling of unusual fatty acids into storage oil is also supported by the results presented by Browse *et al.* and by Zou *et al.*; both have observed an increase of the production of hydroxyl and epoxy fatty acids, respectively, in Arabidopsis. The ricinoleic and vernolic acid contents in *A. thaliana* seeds were raised to around 30% by the co-expression of DAGT2 gene from *Ricinus communis* in *R. communis* FHA12 hydrolase transgenic Arabidopsis, or by DGAT2 gene from *Bernadia pulchella* co-expressed in Arabidopsis transformed by *Crepis palaestina* 12-epoxygase gene. The importance of DAGAT2 in the accumulation of TAG was also point out by Lardizabal *et al.* who observed an increase in seed oil content by 1.5% (by weight) by ectopic expression of an *Umbellopsis* (formerly *Mortierella*) *ramanniana* DGAT2 gene in a stably-inherited phenotype of soybean.

Session 6: Lipid catabolism

Enzymes involved in lipid catabolism play a variety of important physiological roles in plants. Among them is the mobilization of TAG in oilseeds, which occurs following germination and supports early seedling growth, until photosynthetic competence is achieved. Within the oral and poster sessions on lipid catabolism new findings were presented concerning the mechanism of TAG hydrolysis, fatty acid transport, activation and β-oxidation; which are all required for normal seedling establishment in Arabidopsis.

Eastmond *et al.* showed that in Arabidopsis TAG breakdown during early seedling growth can be almost completely blocked by disruption of two TAG lipases, called SDP1 and SDP1L, but while it is not sufficient to prevent seed germination it does impair post-germinative growth. Supplementary data suggest that the activity of these lipases exerts a high degree of control over the rate TAG breakdown in Arabidopsis seedlings.

The catabolism of TAG in Arabidopsis also requires a peroxisomal ABC transporter called CTS (also known as PXA1 or PED3). It is probable that CTS imports either free fatty acids or acyl-CoA into the peroxisome, but its precise substrate specificity is not known. De Marcos Lousa *et al.* presented data showing that the two nucleotide binding domains are functionally distinct and that the substrate selectivity can be altered.



Meeting attendees in the conference room.

Session 7: Lipid signaling and plant responses to stress

The session on lipid signaling covered diverse topics including the formation of lipid signals and their action in the regulation of long-term physiological changes in plants. One common theme was the interaction of lipid signaling pathways with phytohormone signaling in the responses of plants to biotic or abiotic stress. Chapman *et al.* reported that *N*-acylethanolamine (NAE) metabolism – a conserved signaling pathway found in vertebrates - interacted with abscisic acid (ABA) signaling, likely through the activation of ABI3 transcription to mediate growth regulation in response to abiotic stresses. This same pathway interacted with salicylic acid (SA)-mediated signaling in the regulation of plant innate immunity to bacterial pathogens. Additional insights into this NAE regulatory pathway in plants should follow on the heels of the exciting discovery by Faure, Coulon, Bessoule and coworkers, of a novel acyltransferase that produces the NAE precursor, *N*-acyl phosphatidylethanolamine (NAPE).

In other work from Mosblech *et al.* phosphatidylinositide-derived signals were shown to be involved in the modulation of jasmonic acid (JA) signaling and the susceptibility of plants to herbivory. Progress in evaluating short-term lipid signaling processes was presented as well. Munnik described the use of "lipid-biosensors" – lipid-binding protein domains fused to GFP – as subcellular reporters of selective lipid signal formation/metabolism.

Session 8: Lipid trafficking and membrane dynamics

The lipid trafficking between chloroplast and the endoplasmic reticulum (ER) is very important for the development of chloroplasts in plants. C. Benning and co-workers have characterized mutants of Arabidopsis *trigalactosyldiacylglycerol 1, 2, 3, and 4*, in which ER-derived diacylglycerol is not available to galactoglycerolipid biosynthesis and which accumulate oligogalactoglycerolipids and triacylglycerol in their tissues. *TGD4* gene encodes a protein that appears to be associated with the ER membranes and showed a decreased transfer of lipids to isolated plastids indicating a disruption of ER-to-plastid lipid transfer. TGD4 appears to be part of the machinery mediating lipid transfer between the ER and the outer plastid envelope membrane and could be critical in the formation of direct ER-to-plastid envelope contact sites.

Session 9: Modifications of proteins by lipids

Co- and posttranslational modifications are important in the regulation of numerous essential cellular functions. Among them, N-myristoylation is a lipid modification ensuring the proper function and intracellular trafficking of proteins involved in many signaling pathways. T. Meinnel *et al.* presented data strongly suggesting that N-myristoylation is associated with shoot meristem development and that SnRK1 (SNF1-related kinase) is one of its essential primary targets. Using *Arabidopsis thaliana* knockout mutants of a N-myristoyltransferase gene *NMT,* they showed that *NMT1* is strictly required for plant viability and that *NMT1* impairment induced extremely severe defects in the shoot apical meristem during embryonic development, causing growth arrest after germination. Moreover, complementation experiments in the *nmt1-1* background, using either yeast or human NMT, demonstrated a functional link between the developmental arrest of *nmt1-1* mutants and the myristoylation state of an extremely small set of protein targets.

Session 10: New methods and technologies functional lipidomics, fluxome, modeling

Basic methods for lipid analyses (TLC, HPLC, GC) are very limited since they could not separate and quantify the species of the different lipid classes. Recent technologies based on mass spectrometry which allow the quantitative profiling and the structural analysis of select lipid classes have recently merged. C. Ejsing *et al.* from the University of Dresden have developed a novel approach for global, quantitative and molecular characterization of the *Saccharomyces cerevisiae* lipidome. The molecular characterization and absolute quantification of lipid species was made using ion trap-Orbitrap and quadrupole time-of-flight mass spectrometers equipped with automated chip-based nanoelectrospray ionization sources, and data processing by dedicated software applications. By using this technology, they are able to identify 626 different lipid and lipid precursor species.

Session 11: Models for lipid studies – lower plants, micro organisms and others

As lipid peroxidation is common to all biological systems and is regulated by in developmental and environmental processes, many different organisms are used to identify new enzymes and regulation mechanisms. In the moss *Physcomitrella patens* a novel bifunctional LOX which produces octenols from 20:4 and cyclopentenoic fatty acids was identified by I. Feussner *et al.* Cyclopentenoic fatty acids were shown to be involved in sporophyte formation. Moreover, the researchers have characterized in the fungus *Aspergillus nidulans* a new linoleate dioxygenase which synthesizes oxylipins in a comparable reaction catalyzed by a LOX fusion protein from cyanobacteria.

The yeast *Saccharomyces cerevisiae* is also an excellent model for studying lipid metabolism using reverse genetics. T. Czabany *et al.* have identified and characterized two TAG synthases Dga1p and Lro1p, and two steryl esters synthases Are1p and Are2. The study of triple mutants with only one of these acyltransferases active demonstrated that steryl esters form several ordered shells below the surface phospholipid monolayer of lipid particle, whereas TAG are more or less randomly packed in the center of the lipid particle.

A new acyltransferase has been also characterized in the yeast by Le Guédard *et al.* which is required for the incorporation of stearate into PI because GC and LC/MS lipid analyses of KO mutant revealed an almost complete disappearance of 18:0 associated with this phospholipid. The change in the fatty acid composition of PI in mutant cells results from a decrease in the incorporation by acyl-exchange of stearic acid into neo-synthesized molecules of PI (acyl-exchange occur at the *sn*-1 position), rather than from a decrease in the *de novo synthesis* of PI from putative CDP-DAG species containing stearic acid.

 DOI 10.1002/ejlt.200800252 Eur. J. Lipid Sci. Technol. 2008, *110,* 1190–1194

Figure 4 K. Lardizabal, P. Dörmann, A.J. King, J. Browse, J. Jaworski and J. Ohlrogge enjoying the banquet.

Session 12: Plant lipids as renewable sources of energy

Among plants which could be used as an alternative source of biodiesel, *Jatropha curcas* is a promising oilseed crop able to grow easily on various lands. But some varieties actually cultivated produce phorbol esters which are toxic and limit the ability to use the seed-meal as a source for animal feed. I. Graham's team has carried out genotyping and metabolite profiling (oil content, fatty acid profile and phorbol ester analysis) studies in order to identify genetic markers to select Jatropha varieties devoid of phorbol esters.

Enzymes of the lipid metabolism are already important actors of the "green chemistry" and engineering of these proteins will lead to new synthesis processes. U. Bornscheuer presented in his lecture enzymes of the lipid metabolism which are currently used for lipid modification, protein engineering and application of lipases and related enzymes (esterase, phospholipases) as biocatalysts. As an example, the lipase from *Rhizopus oryzae* was modified to increase its stability toward lipid oxidation products such as aldehydes, in order to improve its performance in oleochemical industries.

The 18th ISPL 2008 was also a good opportunity to discover the magnificent city of Bordeaux "a world heritage", to visit famous wineries at Saint-Emilion, to taste the famous wines from Bordeaux area and the French cooking.

The attendees left Bordeaux on Friday afternoon and already thinking ahead to the next ISPL which will be held in Cairns, Australia from 12–16 July 2010.

R. Lessire
Organiser of the 18th ISPL 2008

Additional data and abstracts from the 18th ISPL 2008 are available at: http://www.ispl2008-bordeaux.cnrs.fr/Web^{a}bstracts.pdf

Acknowledgments

The author is grateful to F. Beisson, K. Chapman, J. Dyer and P. Eastmond for their help in preparing this manuscript.

